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The effect of methanol rhizome extract of *Nymphaea lotus* Linn. (Nymphaeaceae) in animal models of diarrhoea

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ABSTRACT

Ethnopharmacological relevance: *Nymphaea lotus*, which is widely distributed throughout tropical Africa, enjoys a number of ethnomedical uses in Nigeria. Traditionally, the rhizomes of *N. lotus* are used to cure diarrhoea.

Aim of study: This study aims to evaluate the antidiarrhoeal activity of the methanol rhizome extract of *N. lotus* plant in laboratory animals.

Materials and methods: The extract was screened for activity against castor oil-induced diarrhoea and magnesium sulphate-induced diarrhoea as well as effect on gastric transit time in mice. The effect of methanol rhizome extract of *Nymphaea lotus* on the perfused isolated tissue preparation was also determined.

Results: For castor oil-induced diarrhoea, the extract at doses of 200, 400 and 800 mg/kg produced significant reduction in the frequency of diarrhoea (at $p < 0.001$, $p < 0.001$ and $p < 0.01$ respectively). The extract at 800 mg/kg produced a significant delay in onset of diarrhoea ($p < 0.05$) comparable to loperamide (3 mg/kg). The frequency of magnesium sulphate-induced diarrhoea was also significantly reduced in the groups treated with 200, 400 and 800 mg/kg of the extract at $p < 0.001$, $p < 0.001$ and $p < 0.01$ respectively. At doses of 200 mg/kg and 400 mg/kg, the protection produced was comparable to loperamide, 3 mg/kg.

All treated groups produced significant reduction in the transit of charcoal meal along the intestinal tract at $p < 0.001$. The extract at low concentration (4×10^{-4}–6.4×10^{-2} mg/ml) had contractile effect on the tone of contraction of the rabbit jejunum while at higher concentrations (8×10^{-2}–512×10^{-2} mg/ml) produced significant reduction in the tone and rate of spontaneous contraction of rabbit jejunum. The extract at lower concentrations (4×10^{-4}–2×10^{-2} mg/ml) has no effect on contraction of the guinea pig ileum while higher concentrations (4×10^{-2}–512×10^{-2} mg/ml) produced significant relaxant activity on guinea pig ileum.

Conclusion: This study has shown that the methanol rhizome extract of *N. lotus* has antidiarrhoeal properties thus justifying its use by the local population for this purpose.

1. Introduction

Diarrhoeal disease account for nearly 1.3 million deaths a year among children under-five years of age, making it the second most common cause of child deaths worldwide (UNICEF, 2012). In Nigeria, it is the number one fatal outcome disease among children under five years of age (Ahmed et al., 2007). Up to 80% of the population in Africa uses traditional medicine to help meet their health care needs (WHO, 2002). It is, therefore, important to identify and evaluate traditional medicine that can be used as alternatives to commonly used antidiarrhoeal drugs, which are often accompanied by adverse effects such as addiction (e.g. diphenoxylate) and constipation (e.g. loperamide) (Hardman and Limbird, 2010). Among these plants, *Nymphaea lotus* Linn, which is widely distributed throughout tropical Africa, enjoys a number of ethnomedical uses in Nigeria (Burkill, 1997). *Nymphaea lotus* rhizome finds applications in the management of circulatory system disorders, digestive system disorders such as diarrhoea, dysentery; genitourinary system disorders as diuretics, mental disorders as insanity, hyposomnia, leprosy, infections and inflammations (Burkill, 1997). Therefore, it is important to establish the scientific basis for the ethnomedical claim of *N. lotus* as an *anti*diarrhoeal agent. This may serve as a source for developing more effective antidiarrhoeal drug.

2. Materials

2.1. Plant material

The rhizomes of *Nymphaea lotus* was obtained from Dogarawa, Zaria Kaduna state. The botanical identification and authentication of the plant was carried out by Mallam Musa Muhammad at the

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herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria where an identification number (894) was obtained.

2.2. Experimental animals

Four male New Zealand rabbits (2–3 kg) and four male guinea pigs (300–500 g) obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria as well as ninety adult Swiss albino mice (20–25 g) of both sexes obtained from National Institute for Trypanosomiasis (and Onchocerciasis) Research (NITR), Kaduna were used for the experiments. The animals were maintained under normal laboratory conditions of humidity, temperature and light for two weeks prior to the experiment so as to allow for their acclimatization. They were provided with commercial rodent diet and water ad libitum. The study was conducted according to ethical guidelines on laboratory animal use and care policy, which is in compliance with Ahmadu Bello University Research Policy (Revised 2010).

2.3. Equipment and other laboratory materials

Microdynamometer (Ugo Basile, Italy); Soxhlet apparatus (Quickfit, England); Water bath (HH-S Digital thermostatic water bath, China); Weighing balance (Lab tech. BL 20,001 and Mettler P162, USA); Cages (locally made for mice); Dissecting kit (Gold Cross Dissecting Set, Malaysia); Data Capsule (Ugo Basile, Italy); Filter papers (Whattman filter papers size 1); Glass wares such as Pipette, Test tubes (Pyrex, France), Syringes (1 ml, 2 ml, 5 ml and 10 ml); Porcelain pestle and mortar; Stop watch.

2.4. Chemicals and drugs

Acacia powder (Evans Medical Lt Speke, Liverpool); Acetylcholine (Sigma-Aldrich Inc., 3050 Spruce Street, St. Louis, USA); Atropine sulphate (Gland Pharma, India); Castor oil (Bell, Sons and Co Ltd, Southport PR9 9 AL, England); Histamine (Sigma-Aldrich Inc., 3050 Spruce Street, St. Louis, USA); Loperamide (Imodium® – Janssen Pharmaceutical, Pakistan); Magnesium sulphate (BDH Chemical Ltd, Poole England); Medicinal charcoal (Ultracarbon® tablets – Merck KGaA, Darmstadt, Germany).

3. Methods

3.1. Preparation of the plant extract

The rhizomes of *Nymphaea lotus* were cleaned and air dried under shade and reduced into a fine powder using mortar and pestle. The powdered rhizome weighing 1.2 kg was extracted using Soxhlet apparatus employing 80% aqueous methanol. The extract was later subjected to drying in flask evaporator under reduced pressure and controlled temperature (40 °C) over a water bath. It was then stored in a levelled air tight container for future use. The weight of the extract obtained was 44.5 g.

3.2. Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening tests for the presence of carbohydrates, anthraquinones, flavonoids, alkaloids, saponins, steroids, tannins, triterpenes and cardiac glycosides using standard test procedures (Evans, 2002; Sofowora, 1993; Silva et al., 2003).

3.3. Acute toxicity studies

The median lethal dose (LD50) of the extract was determined by Lorke's method (1983). The study was carried out in two phases and mice were deprived of food for 12 h prior to administration of the extract. In phase 1, three groups of three mice per group were used. The extract was administered orally in three graded doses (10, 100 and 1000 mg/kg). The treated mice were observed for 4 h post administration for signs of toxicity. After 24 h, no death was recorded, thus phase 2 was initiated. Based on the result in phase 1, three mice were given the extract orally in doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. The mice were then observed for signs of toxicity for the first 4 h and mortality after 24 h.

3.4. In vitro studies

3.4.1. Effect of methanol rhizome extract of Nymphaea lotus on the isolated rabbit jejunum

A New Zealand adult male rabbit used for the study was starved of feed for about 18 h. It was then sacrificed by cervical dislocation. The abdomen was cut open and segments of the jejunum (about 3 cm long) removed and dissected free of adhering mesentery. The tissue was then suspended in a 25 ml organ bath containing tyrode solution and allowed to stabilize for 30 min (period of acclimatization). The effects of acetylcholine (8×10^{-7}–512×10^{-7} mg/ml) and the methanol rhizome extract of *N. lotus* (4×10^{-4}–512×10^{-2} mg/ml) were then tested on the jejunum. The contact time for each concentration was 30 s, which was followed by washing three times with tyrode solution. The tissue was allowed a resting period before addition of the next concentration. The responses were recorded isometrically using a microdynamometer set at a sensitivity of 3.0 mV and a speed of 24 mm/min.

3.4.2. Effect of methanol rhizome extract of Nymphaea lotus on isolated guinea pig ileum

Similar protocol as for that of the effect of methanol rhizome extract of *N. lotus* on isolated rabbit jejunum was followed. The effects of histamine (2×10^{-6}–128×10^{-6} mg/ml) and the methanol rhizome extract of *N. lotus* (4×10^{-4}–512×10^{-2} mg/ml) were then tested on guinea pig ileum.

3.5. Antidiarrhoeal studies

3.5.1. Castor oil induced diarrhoea in mice

The method described by Shoba and Thomas (2001) was used for this study. The mice were fasted for 12 h and divided into five groups of six mice each. Group I received deionized water at a dose of 10 ml/kg p.o. (negative control), Group II received the standard drug loperamide (3 mg/kg p.o.) and served as the positive control while Groups III, IV and V received the methanol rhizome extract of *Nymphaea lotus* at the doses of 200, 400 and 800 mg/kg p.o., respectively. One hour after administration, all mice received 0.5 ml of castor oil p.o and then were placed individually in cages whose floors were lined with pre-weighed white filter paper. During the observation period of 4 h, the time of onset of diarrhoea, frequency of defecation and weight of faeces excreted by the animals were recorded. The percentage protection against diarrhoea was calculated with respect to the number of wet faeces using the formula below:

$$\% \text{ inhibition} = \left[(\text{Number of WFC} - \text{Number of WFT}) / \text{Number of WFC} \right] \times 100\%$$

where;
WFC = wet faeces in negative control group
WFT = wet faeces in test group.